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Comparison of Cellulose Digestion In vivo and In vitro

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COMPARISON OF CELLULOSE DIGESTION

IN VIVO AND IN VITRO

By

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

In vivo digestion trials have been carried out for many years as one means of evaluating animal feeds. The data from these trials have provided the nutritionists with information on feed digestibility. This information has been of great value in determining the feeding value of various feedstuffs and in working out balanced rations for the different classes of livestock. It is generally agreed that *in vivo* digestion trials involving the total collection method provides the most accurate method of determining digestibility of feeds. A serious disadvantage to the method is that the results should be obtained with several animals and they are costly in terms of equipment, time, and labor.

Because of the time consuming and costly nature of the total collection *in vivo* digestion trials, various other methods have been tried to get reliable digestibility data in a faster and less costly manner. One of the more recent methods being used is the 'artificial rumen' (*in vitro*) technique. This method makes use of rumen bacteria and simulated conditions in the rumen of the animal. Cost, time, and labor saving are definitely in its favor. Some of the work reported indicates the *in vitro* technique can be a useful method in nutrition research. Studies on the effect of stage of maturity when harvested on digestibility of forages and the effect of various feed additives on digestibility of roughages have made use of the *in vitro* digestion technique. However, its accuracy and conditions for operation need further testing.

In order for the artificial rumen technique to be used extensively in digestion studies, the results obtained should be comparable to results obtained with the animals. Therefore, the work reported in this thesis was designed to make a series of comparisons of cellulose digestion between the *in vivo* and *in vitro* methods. The specific objectives were the following:

1. To establish the *in vitro* fermentation time which will produce similar cellulose digestion coefficients to those derived *in vivo*.
2. To compare the cellulose digesting ability of cattle and sheep rumen flora *in vitro*.
3. To determine the effect of the ration fed to the animal on the activity of the rumen flora for use in *in vitro* cellulose digestion.
4. To compare the cellulose digestion coefficients from high and low-roughage rations digested both *in vivo* and *in vitro*.
5. To compare buffered rumen fluid with the whole unaltered rumen fluid.
6. To observe the similarity of digestible laboratory nutrients to the total digestible nutrients of a similar ration.

The length of the fermentation time, the cellulose digesting ability of cattle and sheep, the ration effect, and the difference in the method of rumen fluid preparation all are of importance for further study of the artificial rumen technique.

REVIEW OF LITERATURE

The Relationship of *In Vitro* and *In Vivo* Digestion Coefficients

Extensive work has not been reported where digestion coefficients from both animal digestion trials and artificial rumen *in vitro* digestion trials were compared. However, the comparisons which have been made are reported in the following review.

In 1955, Kamstra *et al.* at Ohio were working with the fermentation time *in vitro* needed to correlate with *in vivo* digestion. They found *in vivo* cellulose digestion coefficients of 60.33%, 48.00%, and 31.66%, respectively for orchard grass-alfalfa mixture, a pelleted feed mixture, and a corn and cob meal-poor hay mixture during a twenty-four hour *in vitro* fermentation period. *In vivo* cellulose digestion coefficients from the same feeds with cattle were 68.17%, 46.10%, and 41.61%, respectively. The cellulose coefficients on the first and the last ration *in vitro* were substantially below those *in vivo* indicating that the fermentation period may need lengthening. This is indicated by the work of Pigden and Bell (1955). They demonstrated that total digestible nutrient values obtained after a forty-eight hour fermentation period (*in vitro*) compared favorably with the values obtained with sheep (*in vivo*). Eleven forages were used as substrates in this comparison.

Kamstra (1955) compared digestibility of cellulose in a number of forages by the *in vitro* technique and by digestion trials with sheep. A forty-eight hour fermentation period *in vitro* gave cellulose coefficients similar to those obtained *in vivo*.

A recent study by Ellis et al. (1957) reported the effect of added molybdenum in sheep rations. The in vitro method as well as the digestion trials were used to obtain cellulose digestion coefficients. Both methods of digestion indicated that additional molybdenum increased cellulose degradation by the rumen microorganisms. Lamb gains were stimulated by the addition of molybdenum.

Salasco et al. (1957) experimented with the foliar application of urea to pasture forages. Pongola grass, orchard fescue grass, and prickly pear cactus were the forages used. Bacterial digestion of cellulose was studied both in vitro and in vivo. An increased digestion of cellulose was observed under both methods of digestion when urea had been applied to the forage.

The study of factors has resulted in the extensive use of washed rumen bacteria and purified diets. Whole unaltered rumen fluid and chemically untreated rations were used for the work discussed in this report. Barnett (1957) has discussed these methods. The following quotation from his work supports the use of the untreated constituents in the work reported in this thesis.

> "From the foregoing, the experimenter is led to the inevitable conclusion that despite the interesting work obtained from work with pure cultures or washed suspensions and with chemically prepared fiber or cellulose, the results most akin to the in vivo ones are likely to be attained in vitro by the use of chemically untreated feeding stuff and whole filtered rumen liquor, always on the assumption that the operative microflora of the latter may be kept sufficiently active for a suitable length of time."

In Barnett's work, digestion coefficients for crude fiber of silage calculated from in vivo digestion trials were closely correlated with cellulose digestion coefficients derived from in vitro

digestion. Whole filtered rumen fluid was used as innoculum for *in vitro* digestion. The fermentation time used for the *in vitro* digestion trials was seventy hours. A cellulose digestion curve revealed maximum digestion near the fifty hour fermentation period. The average cellulose digestibility values were plotted against the corresponding crude fiber digestibility results. The correlation was reasonably good when this simple equation was used: crude fiber digestion coefficients (*in vivo*) = cellulose digestion coefficients (*in vitro*) + 2.9.

The limited number of experiments which have been published seems to justify further experiments comparing *in vitro* and *in vivo* digestion coefficients. No work directly related to objectives three, four, five, and six was available. These objectives are of prime importance for further use of the artificial rumen technique.

Comparing the Digestive Ability of Cattle and Sheep

Objective two was to compare the cellulose digesting ability of cattle and sheep rumen flora *in vitro*. No previous *in vitro* work was available although there are many *in vivo* comparisons of the digestive powers of sheep and cattle. This section of the review of literature covers the *in vivo* experiments.

There has been considerable controversy over the comparative digestive powers of sheep and cattle. Work carried on by Neidig *et al.* (1921), Lathrop *et al.* (1938), and Forbes (1950) indicated that under the conditions of their experiments sheep tended to be somewhat more efficient in feed digestion than cattle. On the other hand, Frear (1890) concluded that the digestive power of sheep under the conditions of his trials was inferior to that of steers. Other studies reported by Watson *et al.* (1948), Cippelloni *et al.* (1951), and Jordon and Staples (1951) showed similarity in the results between the two species. It was also expressed that for greatest accuracy for a particular species, digestibility data must be obtained from that species.

Rumen bacteria of cattle and sheep on practical winter rations and pasture were studied by Gall *et al.* (1949). The bacteria were studied by means of direct slide counts, gram stains, and anaerobic cultural techniques. The bacterial pictures, as seen on gram stains from cattle and sheep on several winter rations, resembled each other closely. The bacteria from animals on pasture appeared morphologically similar to those on winter rations; however, a few different types of bacteria were seen in addition to those on the winter rations.

Cultures from both species on all rations presented a rather uniform pattern in many respects; although, there was a noticeable increase in fast growing organisms correlated with an increase in the amount of grain in the ration. Thus, cattle and sheep did not appear to have a markedly different bacterial population when fed similar rations.

This review did not show a definite advantage of increased digestion when either cattle or sheep were used. Any differences between cattle and sheep rumen fluid should be known because both are a source of innoculum for the artificial rumen. Since no *in vitro* experiments comparing cattle and sheep digestive powers are available, a comparison of this type is of consequence.

METHOD OF PROCEDURE

In Vitro Rumen Fermentation Procedure

The *in vitro* fermentation experiments for obtaining cellulose digestion coefficients were carried out according to the method of Burroughs *et al.* (1950c) as modified by Bentley *et al.* (1954).

The individual flasks were placed in a thermostatically controlled water bath (38.0°C. ± 0.2°C.) and individually gassed with carbon dioxide as pictured in Figure 1. The rubber stoppers were fitted with two glass tubes; one going below the surface of the medium through which the carbon dioxide entered, and the other being a short tube through which the gas escaped.

Adjustments to the pH, 6.8, were made at four-hour intervals during the first twelve hours of fermentation and every ten to twelve hours thereafter. A saturated solution of Na_2CO_3 was added to adjust the pH.

At the termination of the fermentation period, the flask contents were diluted to one hundred milliliters with distilled water. Duplicate ten milliliter aliquots were taken for cellulose analysis after the flasks contents were thoroughly mixed.

The composition of the basal medium, which was used in all *in vitro* fermentation trials, is listed in Table 1. The rumen flora suspension was not added until the water bath temperature was optimum and the flasks had been thoroughly gassed with CO_2 from ten to fifteen minutes. Carbon dioxide was bubbled through the flask slowly throughout the entire fermentation period to maintain anaerobic conditions, yet

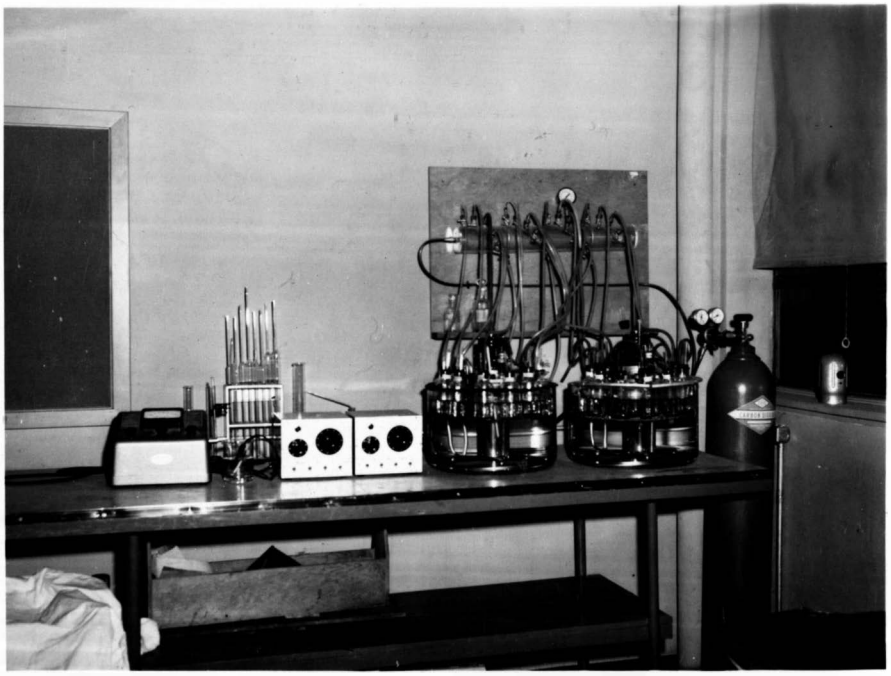

Figure 1. Apparatus utilised for *in vitro* digestion.

Table 1. Composition of Basal Medium for the In Vitro Rumen Fermentation

Constituent	Grams/100 Milliliters
Ration	2.0
Na_2HPO_4	0.113
NaH_2PO_4	0.109
KCl	0.043
NaCl	0.043
$MgSO_4 \cdot 7H_2O$	0.01164
Na_2SO_4	0.015
$FeCl_3$	0.00064
	Milliliters/100 Milliliters
Buffer pH 6.3[1]	15.0
Inoculum[2]	40.0

[1] Milliliters of each constituent added to form buffer: .154 M $(NH_4)_2HPO_4$; 5 ml.: .154 M $MgSO_4$; 1 ml.: .2 M $NaHCO_3$; 100 ml.

[2] Whole rumen fluid strained through four layers of cheesecloth.

not so briskly as to result in lowered digestion. Hallilwell (1957) has shown that excessive bubbling may decrease cellulose digestion.

The rumen fluid was obtained from the animal and strained through four layers of cheesecloth. This was the only preparation before addition to the fermentation flasks. Cellulose analysis of the rumen fluid was obtained so as to increase the accuracy of the in vitro cellulose coefficients. The forty milliliters of rumen fluid used for innoculation consisted of microörganisms, protozoa, and small plant particles.

All rations were thoroughly mixed, dried, and ground through a forty mesh screen before being added to the ferawntation flask. If a coarser grind was used, plugging of the pipette occurred.

Source of Rumen Flora

The rumen fluid was obtained for these digestion experiments from grade Hereford twin steers. A rumen fistula had been made in one of these steers at the weight of approximately four hundred pounds. This steer developed poorly for the next eight months. The poor growth and condition of this steer were due to the loss of rumen contents through the fistula. An inflated plug was used which proved unsatisfactory. It was later changed to an uninflated type rumen plug, and the steer has done very well with little loss of the rumen contents since that time.

The second twin was fitted with a rumen cannula at a later date. The cannula left the rumen fluid readily available but it did not give access to the rumen that the rumen fistula offered. Wether feeder lambs, predominantly of Rambouillet breeding, also were fitted with rumen cannulas for the source of sheep rumen fluid. Figures 2 and 3 illustrate the cannulated animals. Cannulation has proved very satisfactory with both steers and sheep. Close attention should be given during the summer months to prevent infestation by maggots.

The wethers and steers were maintained on a similar control ration of alfalfa hay, rolled shelled corn, and soybean oil meal. The wethers received eighty grams of rolled corn, forty grams of soybean oil meal, and three hundred grams of alfalfa hay twice daily. The steers were fed eight hundred grams of rolled corn, four hundred grams of soybean oil meal, and three thousand grams of alfalfa hay twice daily. The alfalfa hay was of medium quality. Trace-mineralized salt

Figure 2. A view of the cannulated steer.

Figure 3. A view of a cannulated sheep.

and clean water were available free choice. The animals were quartered in the animal husbandry nutritional research building at all times. The steers were kept tied or stanchioned. Separate pens were used for the sheep (Figure 4).

Rumen fluid was taken from the cannulated animals with a Thompson suction pump fitted with a suction flask (Figure 5). The rumen fluid was then strained through four layers of cheesecloth before being added to the digestion bottles. Because of the ease of collection, this method was used predominantly. When rumen fluid from the fistulated animal was obtained, a more lengthy procedure was carried out. The rumen contents were scooped out of the rumen onto eight layers of cheesecloth as demonstrated in Figure 6. The fluid was then removed by squeezing it in a lard press (Figure 7). Before innoculating the digestion flasks, the rumen fluid was again strained through four layers of cheesecloth. The rumen fluid, after this preparation, is commonly referred to as 'whole filtered rumen fluid'.

Figure 4. A view of the pens housing the cannulated sheep.

Figure 5. The Thompson suction pump fitted with a suction flask.

Figure 6. Collecting rumen contents from a fistulated steer.

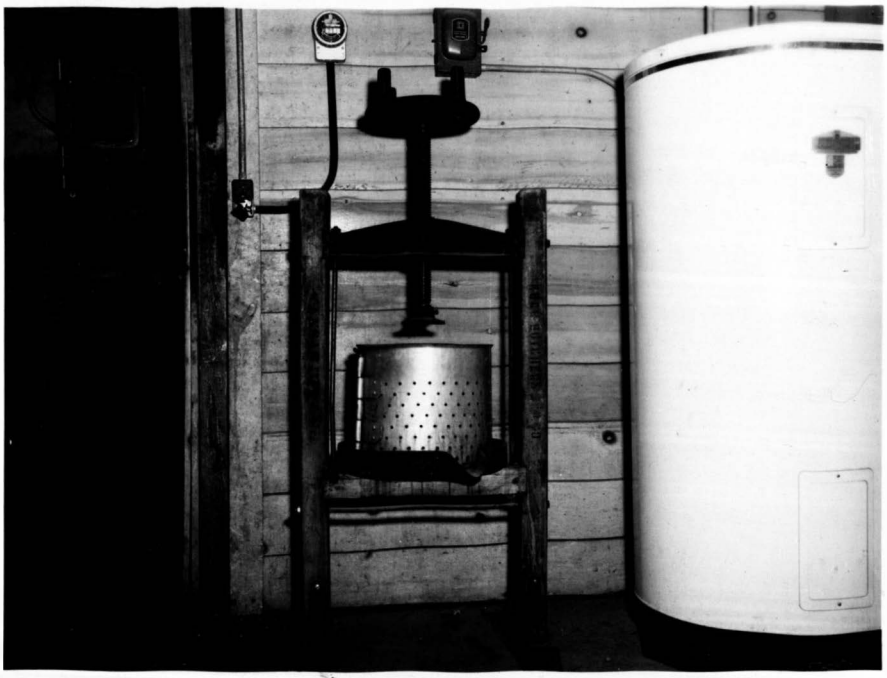

Figure 7. The press used for removing rumen fluid from the solid material.

In Vivo Cellulose Digestion Trials

The samples of feed, feces, and orts were available from previously conducted digestion trials. They were analyzed for cellulose content by the method of Crampton and Maynard (1938). A comparison of *in vivo* and *in vitro* cellulose digestion coefficients were made on twenty-two different rations. Table 2 lists these rations and their cellulose content. The rations were of several types: alfalfa silage, alfalfa-oats mixture, and prairie hay. Penicillin, a chemobiotic (Dynafac), and a dried rumen product (Rufus), were added to the alfalfa-oats ration. Corn, sulphuric acid, and a dried molasses product (Car-mo-las) were used as preservatives in the alfalfa silage. The protein of the prairie hay ration was increased to ten per cent by the addition of soybean oil meal.

Table 2. Rations Utilized for *In Vitro* and *In Vivo* Digestion

Number	Ration	Percentage Cellulose
1	Alfalfa silage (no preservative added)	30.39
2	Alfalfa silage (15 lb. corn per 100 lb. fresh forage)	28.26
3	Alfalfa silage (25 l. of 2.0 N H_2SO_4 per ton)	31.98
4	Alfalfa silage (5 lb. Car-mo-las per 100 lb. fresh forage)	30.92
5	75% oats, 25% alfalfa hay	17.21
6	75% oats, 25% alfalfa hay + penicillin, 10 mg. per lb.	16.97
7	75% oats, 25% alfalfa hay + Dynafac, ½ g. per lb. oats	16.89
8	75% oats, 25% alfalfa hay + Rufus, ½ g. per lb. oats	16.29
9	25% oats, 75% alfalfa hay	20.05
10	25% oats, 75% alfalfa hay + penicillin, 10 mg. per lb.	19.79
11	25% oats, 75% alfalfa hay + Dynafac, 1½ g. per lb. oats	20.02
12	25% oats, 75% alfalfa hay + Rufus, 1½ g. per lb. oats	19.36
13	Prairie hay stored in the stack + SBOM to increase protein to 10%	32.91
14	Prairie hay left in the field until May + SBOM to increase protein to 10%	34.29
15	Prairie hay left in the field until Oct. + SBOM to increase protein to 10%	33.67

Table 2. Rations Utilized for *In Vitro* and *In Vivo* Digestion (continued)

Number	Ration	Percentage Cellulose
16	Prairie hay stored in the nutrition lab. + SBOM to increase protein to 10%	34.81
17	Prairie hay cut in 1947 + SBOM to increase protein to 10%	36.50
18	Prairie hay cut in 1948 + SBOM to increase protein to 10%	35.53
19	Prairie hay cut in 1952 + SBOM to increase protein to 10%	38.06
20	Prairie hay cut in 1953 (hauled in in 1954) + SBOM to increase protein to 10%	34.51
21	Prairie hay cut in 1953 (hauled in in 1957) + SBOM to increase protein to 10%	37.89
22	Prairie hay cut in 1954 + SBOM to increase protein to 10%	36.04

Methods of Calculating Cellulose Digestion Coefficients

After the cellulose analysis had been completed on the various samples from the digestion trials, cellulose digestion coefficients were calculated. The following series of formulas resulted in the per cent cellulose digestion:

1. Dry matter x per cent cellulose = cellulose (ration, orts, feces).
2. Cellulose ration - cellulose orts = cellulose intake.
3. Cellulose intake - cellulose feces = cellulose digested.
4. Cellulose digested ÷ cellulose intake x 100 = per cent cellulose digestion in vivo.

The figures were all in grams on the dry-matter basis.

The in vitro cellulose digestion coefficients were calculated by the following series of formulae:

1. Cellulose/gm. ration x 2 gm. ration = total cellulose.
2. Cellulose/10 ml. aliquot x 10 = non-digested cellulose.
3. Non-digested cellulose - cellulose/40 ml. rumen flora = corrected non-digested cellulose.
4. Total cellulose - corrected non-digested cellulose = cellulose digested.
5. Cellulose digested ÷ total cellulose x 100 = per cent cellulose digestion.

RESULTS AND DISCUSSION

Comparison of *In Vitro* and *In Vivo* Cellulose Digestion Coefficients

The main objective of this thesis was to compare *in vitro* and *in vivo* cellulose digestion coefficients in different ways. The first comparisons were made to establish the length of time necessary for *in vitro* fermentation so the resulting cellulose digestion would be similar to cellulose digestion *in vivo*. During preliminary trials, the fermentation time ranged from eight to forty-eight hours. The shorter length of fermentation generally resulted in less cellulose digestion than that obtained *in vivo*. The results from forty-eight hours *in vitro* cellulose digestion were similar to those of the *in vivo* digestion. After careful consideration of the data from the preliminary trials, twenty-four and forty-eight hour fermentation periods were used in comparison to the *in vivo* coefficients.

The fermentation systems were operated in the manner described by Bentley *et al.* (1954). Both twenty-four and forty-eight hour aliquots were taken from the same fermentation flask. Cellulose analysis and coefficients were obtained in the manner described previously. From digestion trials with sheep, *in vivo* cellulose digestion coefficients were calculated. The rumen fluid used as inoculum in the *in vitro* digestion flasks was also obtained from sheep. The time interval between *in vitro* and *in vivo* digestion trials ranged from a few days up to nine months.

All twenty-two rations (Table 2) were used for these comparisons. The data are presented in Table 3. The *in vitro* cellulose coefficients

were derived from an average of the analysis of four 10 ml. aliquot samples. Two *in vitro* digestion trials were carried out with each ration and duplicate aliquots were taken from each trial. The *in vivo* cellulose coefficients represented the average values from three to eight sheep. The exact number of coefficients averaged to obtain the *in vivo* coefficient in Table 3 were synonymous with the column 'sheep in trial'.

The means for twenty-four hour *in vitro*, forty-eight hour *in vitro*, and *in vivo* cellulose digestion coefficients were 31.62, 50.17, and 45.80, respectively. The t-test was used as the method of statistical analysis. There was a statistically significant difference between the twenty-four hour *in vitro* and the *in vivo* cellulose digestion coefficients. No significant difference between the forty-eight hour *in vitro* and the *in vivo* cellulose digestion coefficients occurred. The correlation between the forty-eight hour *in vitro* and the *in vivo* cellulose digestion coefficients was .307 ($P<0.05$).

It was quite evident that *in vivo* and *in vitro* cellulose coefficients from the last six prairie hay rations, seventeen through twenty-two as listed in Table 2, demonstrated little relationship. As may be noted, the rations seventeen through twenty-two (Table 2) were similar and cellulose digestion coefficients were obtained from the same digestion trials. There was no reasonable explanation for the large difference between the coefficients. Yet, it seemed of interest to run a correlation without these last six rations. When the first sixteen rations were used in the statistical correlation, the correlation was .841 ($P<0.01$).

Table 3. Comparison of *In Vitro* and *In Vivo* Cellulose Digestion Coefficients

Ration	Twenty-four Hour In Vitro Digestion		Forty-eight Hour In Vitro Digestion		In Vivo Digestion	
	Number of Samples	Average Cellulose Coefficient	Number of Samples	Average Cellulose Coefficient	Sheep in Trial	Cellulose Coefficient
1	4	28.86	4	40.43	3	51.10
2	4	33.08	4	40.91	3	60.34
3	4	28.88	4	59.05	3	61.09
4	4	28.61	4	44.62	3	48.24
5	4	22.05	4	40.50	4	34.47
6	4	10.35	4	28.76	4	31.71
7	4	24.43	4	32.27	4	27.03
8	4	18.37	4	24.86	4	29.54
9	4	29.85	4	44.81	4	47.72
10	4	41.67	4	50.03	4	46.50
11	4	24.75	4	42.98	4	45.95
12	4	30.06	4	45.05	4	45.39
13	4	28.78	4	56.43	8	59.44
14	4	40.76	4	55.27	8	56.15

Table 3. Comparison of *In Vitro* and *In Vivo* Cellulose Digestion Coefficients (continued)

Ration	Twenty-four Hour In Vitro Digestion: Number of Samples	Twenty-four Hour In Vitro Digestion: Average Cellulose Coefficient	Forty-eight Hour In Vitro Digestion: Number of Samples	Forty-eight Hour In Vitro Digestion: Average Cellulose Coefficient	In Vivo Digestion: Sheep in Trial	In Vivo Digestion: Cellulose Coefficient
15	4	23.63	4	52.37	8	55.62
16	4	30.08	4	49.98	8	58.55
17	4	39.51	4	64.64	3	26.38
18	4	42.08	4	64.88	3	37.12
19	4	46.92	4	65.51	3	47.32
20	4	38.12	4	65.65	3	40.55
21	4	47.40	4	70.89	3	50.96
22	4	37.00	4	63.78	3	47.69
Mean		31.62		50.17		45.86

The Comparative Digestive Power of Sheep and Steer Rumen Flora *In Vitro*

Sheep and steer rumen fluid were both being used as innoculum for *in vitro* cellulose digestion. Thus, a comparison of their cellulose digestive powers seemed desirable. *In vitro* digestion trials were run using twenty-two rations (Table 2). The procedure was the same in all trials with the exception of the source of rumen fluid used as innoculum. Rumen fluid from both the sheep and the steers was obtained the same day for digestion of the same ration. Ten milliliter aliquots, in duplicate, were taken from each digestion flask at twenty-four and forty-eight hour intervals. Two replications of each digestion trial were run.

In vitro cellulose digestion coefficients using sheep and steer rumen innoculum at twenty-four and forty-eight hour intervals were compared (Table 4). The means of twenty-two *in vitro* cellulose digestion coefficients at a twenty-four hour fermentation period using sheep and steer rumen fluid as innoculum were 31.61 and 30.81, respectively. The means of twenty-two *in vitro* cellulose digestion coefficients from forty-eight hour fermentation using sheep and steer rumen fluid as innoculum were 50.17 and 49.98, respectively. The correlation between the steer and the sheep cellulose digestion coefficients from the twenty-four hour fermentation period was .778 ($P<0.01$). The correlation between the steer and the sheep cellulose digestion coefficients from the forty-eight hour fermentation period was .918 ($P<0.01$). These correlations further support the use of a forty-eight hour fermentation period.

The means and correlation coefficients from this trial indicated

very little difference in *in vitro* cellulose digestion at twenty-four and forty-eight hour fermentation periods when rumen fluid for inoculum was obtained from either steers or sheep.

Table 4. Correlation Between Sheep and Cattle Rumen Flora Cellulose Digestion

Ration	Replications	Twenty-four Hour In Vitro Cellulose Coefficient		Forty-eight Hour In Vitro Cellulose Coefficient	
		Sheep	Steer	Sheep	Steer
1	2	28.86	26.98	40.43	40.01
2	2	33.08	39.32	40.91	46.83
3	2	28.88	33.02	59.05	46.46
4	2	28.61	29.92	44.62	46.70
5	2	22.05	4.75	40.50	28.72
6	2	10.35	2.65	28.76	16.66
7	2	24.43	11.24	32.27	24.26
8	2	15.73	1.98	24.86	27.14
9	2	29.85	40.26	44.81	46.50
10	2	41.57	39.10	50.03	52.23
11	2	24.75	37.54	42.98	48.04
12	2	30.06	39.43	45.05	49.63
13	2	28.78	28.92	56.43	51.79
14	2	40.76	29.01	55.27	58.91

Table 4. Correlation Between Sheep and Cattle Rumen Flora Cellulose Digestion (continued)

Ration	Replications	Twenty-four Hour In Vitro Cellulose Coefficient		Forty-eight Hour In Vitro Cellulose Coefficient	
		Sheep	Steer	Sheep	Steer
15	2	23.63	34.31	52.37	55.99
15	2	30.08	33.66	49.98	58.37
17	2	39.51	42.32	64.64	64.44
18	2	42.08	37.02	64.88	64.66
19	2	46.92	43.24	65.51	67.34
20	2	38.12	37.33	65.65	64.56
21	2	47.40	49.71	70.89	69.58
22	2	37.00	36.17	63.78	61.79
Mean		31.61	30.81	50.17	49.58

Effect of the Previous Ration on Cellulose Digesting Ability of Rumen Flora

This trial was run to evaluate the difference in the cellulose digestive powers of rumen flora from sheep fed different rations. To define this further, cannulated sheep were fed the ration that was to be digested *in vitro* and rumen fluid from these sheep was used as inoculum. Rumen fluid from cannulated sheep on another ration, which will be designated as 'control', was also used as inoculum. The cellulose digestive ability of these two inoculum sources was checked on the test rations.

Rations six, seven, and eight (Table 2) were used as the rations for *in vitro* digestion and were fed to sheep from which the rumen fluid was obtained for cellulose digestion. Ration five (Table 2) was also fed to sheep which were used as a source of rumen fluid. Each of the test rations (six, seven, and eight) were digested *in vitro* by rumen fluid obtained from sheep fed the particular test ration and another ration (the control). In this way a comparison could be made of the importance of obtaining the rumen fluid from an animal being fed the ration to be tested.

All data from this comparison are given in Table 5. Cellulose digestion coefficients were obtained for four different fermentation times; fifteen, eighteen, twenty-one, and twenty-four hours. 'Rumen fluid control', refers to that taken from sheep on the control ration or ration five. 'Rumen fluid as fed', implies that the rumen fluid was taken from a sheep being fed the ration tested. The *in vitro* cellulose digestion coefficients means for the 'control' and 'as fed' rumen

fluid were 42.35 and 36.06, respectively.

A t-test was applied as a means of statistical analysis. There was a significant difference between the cellulose digesting ability of the two sources of rumen fluid ($P<0.01$). The control rumen fluid showed the higher cellulose digestion coefficients in most cases. An increase in fermentation time resulted in increased cellulose digestion which was similar with all rations and with both sources of rumen fluid.

Table 5. Cellulose Digestive Powers of Rumen Flora from Sheep on a Control Ration Compared to Rumen Flora from Sheep on the Test Ration

Ration	Hours of Fermentation	Rumen Flora	
		Control[3]	As Fed[4]
6	15	33.74	22.96
	18	39.30	28.85
	21	40.31	37.61
	24	49.40	43.17
Average		40.91	33.15
7	15	35.98	21.33
	18	41.97	38.48
	21	44.47	45.14
	24	49.13	46.47
Average		42.89	37.86
8	15	33.25	33.59
	18	39.79	32.21
	21	43.92	37.21
	24	56.49	45.64
Average		43.36	37.19
Mean		42.35	36.06

[3] Rumen flora obtained from sheep on the control diet.

[4] Rumen flora obtained from sheep on the same diet as was digested *in vitro*.

The Effect of High and Low-Roughage Rations on Cellulose Digestion *In Vitro* and *In Vivo*

This experiment was to test the effect of high and low-roughage rations on the extent of cellulose digestion *in vitro* and *in vivo*. It had been noted by Burroughs *et al.* (1949b), Swift *et al.* (1947), Arias *et al.* (1951), and Head (1953) that an increase in the amount of starch in the ration may decrease the cellulose digestion. Low-roughage rations used for digestion in this trial were rations five, six, seven, and eight listed in Table 2. High-roughage rations used for digestion in this trial were rations nine, ten, eleven, and twelve listed in Table 2.

The comparisons were made in three different units: forty-eight hour cellulose digestion coefficients *in vitro* using sheep rumen fluid; forty-eight hour cellulose digestion coefficients *in vitro* using steer rumen fluid; and *in vivo* cellulose digestion coefficients obtained from sheep digestion trials. The data from these digestion trials are given in Table 6.

These high and low-roughage rations were first compared *in vitro* with a forty-eight hour fermentation period using sheep rumen fluid as inoculum. Each cellulose digestion coefficient was derived from four separate cellulose analysis as described for previous trials. The means of the cellulose digestion coefficients from the low and high-roughage rations were 31.60 and 45.72, respectively. The t-test demonstrated a significant difference between the cellulose coefficients from the high and low-roughage rations ($P<0.05$). The larger cellulose coefficients resulted from *in vitro* digestion of the higher roughage

ration.

The second comparison of this experiment was made under similar conditions to the first comparison except that steer rumen fluid was used as the source of the innoculum. The mean from the low-roughage ration cellulose digestion coefficients was 24.20, while the mean from the high-roughage cellulose digestion coefficients was 49.10. The t-test was used as a means of statistical analysis, resulting in $P<0.01$. The larger cellulose digestion coefficients were obtained from digestion of the high-roughage rations.

The third comparison within this experiment was again with the same high and low-roughage rations but the cellulose digestion coefficients were derived from *in vivo* digestion trials with sheep. Four sheep were placed on each ration and an average cellulose digestion coefficient was derived. The means for the high and low-roughage rations were 46.39 and 30.69, respectively. The t-test was used as a means of statistical analysis, resulting in $P<0.01$. The high-roughage ration gave larger cellulose digestion coefficients than the low-roughage ration.

All units of this comparison demonstrated that cellulose coefficients from high-roughage rations were greater than cellulose coefficients from low-roughage rations. Similar results were obtained from both *in vitro* and *in vivo* cellulose digestion.

Table 6. Comparing Cellulose Digestion in High and Low-Roughage Rations[5]

	Sheep Rumen Fluid Used as Innoculum for Forty-eight Hour In Vitro Cellulose Digestion		
Ration Number	Low-Roughage	Ration Number	High-Roughage
5	40.50	9	44.81
6	28.76	10	50.03
7	32.27	11	42.98
8	24.86	12	45.05
Mean	31.60		45.72

	Steer Rumen Fluid Used as Innoculum for Forty-eight Hour In Vitro Cellulose Digestion		
Ration Number	Low-Roughage	Ration Number	High-Roughage
5	28.72	9	46.50
6	16.66	10	52.23
7	24.26	11	48.04
8	27.14	12	49.63
Mean	24.20		49.10

[5]High-roughage ration, 25% oats + 75% alfalfa hay. Low-roughage ration, 75% oats + 25% alfalfa hay.

Table 6. Comparing Cellulose Digestion in High and Low-Roughage Rations (continued)[5]

Sheep In Vivo Cellulose Digestion			
Ration Number	Low-Roughage	Ration Number	High-Roughage
5	34.47	9	47.72
6	31.71	10	46.50
7	27.03	11	45.95
8	29.54	12	45.39
Mean	30.69		46.39

[5] High-roughage ration, 25% oats + 75% alfalfa hay. Low-roughage ration, 75% oats + 25% alfalfa hay.

Unaltered Rumen Fluid Compared to Rumen Fluid Derived from Rumen Cake

Johnson at al. (1957) reported a method of preparing rumen fluid that increased in vitro cellulose digestion. The rumen contents were taken from the fistulated animal and the liquid removed by means of the press described previously. Then, fifty grams of the fibrous cake were suspended in one hundred milliliters of isotonic phosphate buffer. The liquid was again removed from the fibrous material with a Carver hydraulic press at one hundred and thirty pounds pressure per square inch. The liquid removed from the last operation was used as innoculum.

This experiment was carried on to compare in vitro cellulose digestion from the above method of innoculum preparation and the previous method of preparing innoculum in an effort to reduce variation between trials. Rations five, six, nine, and ten (Table 2) were used for this study. Rations five and six were low-roughage rations, while nine and ten were high-roughage rations. Duplicate ten milliliter aliquots of digested substrate for cellulose analysis were taken at twenty-four and forty-eight hour fermentation periods. Three replications of the in vitro digestion trial were run during three consecutive weeks. 'Cake and buffered' refers to the method of innoculum preparation described above. 'Whole rumen fluid' refers to the previous method of preparation.

The results of this experiment are shown in Table 7. The data were analyzed by an analysis of variance. The method x replications interaction resulted in a non-significant difference, indicating little difference in the cellulose digestive powers of rumen fluid prepared

by these methods and fermented under these conditions. Also, it was evident that no reduction in the variation between trials had occurred.

Table 7. Whole Unaltered Rumen Fluid as Compared to Caked and Buffered Rumen Fluid

Caked and Buffered Rumen Fluid for Twenty-four Hour *In Vitro* Digestion			
Ration	Replication 1	Replication 2	Replication 3
5	0.00	1.07	9.73
6	12.37	10.87	17.00
9	29.08	34.29	44.21
10	37.49	33.23	42.98

Caked and Buffered Rumen Fluid for Forty-eight Hour *In Vitro* Digestion			
Ration	Replication 1	Replication 2	Replication 3
5	15.05	62.38	26.58
6	13.85	47.70	32.61
9	39.05	56.98	48.07
10	50.02	64.25	51.81

Whole Rumen Fluid for Twenty-four Hour *In Vitro* Digestion			
Ration	Replication 1	Replication 2	Replication 3
5	0.06	10.52	7.26
6	5.13	9.55	13.91
9	32.17	34.16	44.59
10	34.06	37.09	50.93

Table 7. Whole Unaltered Rumen Fluid as Compared to Caked and Buffered Rumen Fluid (continued)

Whole Rumen Fluid for Forty-eight Hour In Vitro Digestion			
Ration	Replication 1	Replication 2	Replication 3
5	14.58	38.41	24.99
6	1.30	40.19	36.89
9	29.68	55.61	45.84
10	43.91	54.02	51.44

Analysis of Variance Table from Rumen Fluid Preparation Comparisons			
Source	Degrees of Freedom	Mean Square	F-value
Total	48		
Methods	1	100.63	< 1
Replications	2	1,051.44	3.49[6]
Replications x method	2	13.52	< 1
Pooled errors	43	300.99	

[6] Significant at the five per cent level of probability.

Digestible Laboratory Nutrients as a Means of Feed Evaluation

Digestible laboratory nutrients were calculated for each type of ration given in Table 2 by the Agricultural Biochemistry Department. The method used has been described by Thurman and Wehunt (1955). These figures were given along with TDN values, *in vivo* cellulose digestion values, and per cent cellulose (Table 8). The TDN values were calculated using Morrison's 'Feeds and Feeding', 22nd edition. The TDN values used were from alfalfa hay (all analyses), oats (not including Pacific Coast States), alfalfa silage (no preservative), and alfalfa-molasses silage. The *in vivo* cellulose coefficients and per cent cellulose of these rations were taken from previous data in this thesis.

It was interesting to note the favorable comparison between the digestible laboratory nutrient figures and the *in vivo* cellulose coefficients of the prairie hay and alfalfa silage rations. Also of interest was that as the cellulose content of the ration decreased, the TDN values as well as the DLN values increased. With the five rations analyzed in this comparison, digestible laboratory nutrients seemed to show some promise as a method of feed evaluation when compared to total digestible nutrients and *in vivo* cellulose digestion coefficients.

Some constituents of a ration which affect their digestibility may not be reflected by a chemical method of feed evaluation. Additives such as antibiotics, chemobiotics, and dried rumen products would fall into this category. Also, palatability could not be reflected by a laboratory method of feed evaluation. Thus, even though

there may be good comparisons between chemical and animal methods of feed evaluation under some conditions, the chemical methods should not be used without giving consideration to such factors as those listed above.

Table 8. Digestible Laboratory Nutrients as a Method of Feed Evaluation

Ration	Per Cent Cellulose	Cellulose Digestion In Vivo	Per Cent DLN	Per Cent TDN
Alfalfa silage, no preservative	30.89	51.10	52.07	54.0
Alfalfa silage + molasses	30.92	48.24	49.82	57.4
Prairie hay	34.29	56.15	52.30	49.6
75% alfalfa[7], 25% oats[8]	20.05	47.72	71.94	61.0
25% alfalfa[7], 75% oats[8]	17.21	34.47	73.04	71.8

[7] Alfalfa hay, all analysis.

[8] Oats, not including Pacific Coast States.

SUMMARY AND CONCLUSIONS

Work was carried on comparing *in vitro* and *in vivo* cellulose digestion. *In vivo* cellulose digestion was determined with sheep on digestion trials and *in vitro* cellulose digestion by the use of the artificial rumen technique. Twenty-two rations were used for cellulose digestion.

The primary objective was to establish an *in vitro* fermentation interval that would result in cellulose digestion comparable to cellulose digestion *in vivo*. In preliminary trials, the fermentation time ranged from eight to forty-eight hours. After due consideration, cellulose digestion at twenty-four and forty-eight hour intervals was compared to cellulose digestion *in vivo*. Cellulose coefficients derived from twenty-four hour *in vitro* digestion varied considerably from *in vivo* digestion. The forty-eight hour fermentation period produced cellulose digestion similar to the cellulose digestion *in vivo*.

Per cent cellulose digestion *in vitro* using sheep rumen fluid and steer rumen fluid was compared. When twenty-two rations were used, the correlation between the two sources of rumen fluid was statistically significant ($P<0.01$). Indications were that there was little difference in the amount of cellulose digestion *in vitro* when steers or sheep were used as a source of rumen fluid.

The effect of an animal's ration on the cellulose digesting ability of its rumen flora was determined. There was no advantage in cellulose digestion *in vitro* by taking the innoculum from an animal fed the particular ration being tested over innoculum taken from an animal on

a different diet.

A definite trend indicating greater cellulose digestion in high-roughage rations as compared to low-roughage rations was established in three comparisons. In all three trials, sheep and steer rumen flora in vitro and sheep in vivo digestion trials resulted in significantly greater cellulose digestion in the high-roughage rations.

An alternative method of rumen fluid preparation was used in an attempt to minimize variation between in vitro trials. When a comparison was made between the caked and buffered rumen fluid and the whole unaltered rumen fluid, there was no significant difference in the variation between trials or in the amount of cellulose digested.

Observations of another method of feed evaluation, digestible laboratory nutrients (DLN), showed promise when compared to total digestible nutrients (TDN). It was evident that as the cellulose content of the ration increased, both digestible laboratory nutrients and total digestible nutrients decreased. Since only five types of rations were used in this comparison, no definite conclusions can be drawn. However, certain factors could affect digestibility and palatability of the ration which would have no influence on the laboratory method of feed evaluation.

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